

ETS1 suppresses Tumorigenesis of Human Breast Cancer via Trans-Activation of Canonical Tumor Suppressor Genes

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Supplementary Information

Supplementary Figure 1. Comparison of *ETS1* expression between normal and tumor patient specimens in publically available dataset (Curtis).

Supplementary Figure 2. Comparison of *ETS1* expression between normal and tumor patient specimens in diverse cancer types.

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Supplementary Table 1. Primer sequences used for qRT-PCR

Supplementary Table 2. Primer sequences used for ChIP-PCR.

Supplementary Table 3. Correlation between methylation value of CpG site and *ETS1* expression in normal and tumor specimens.

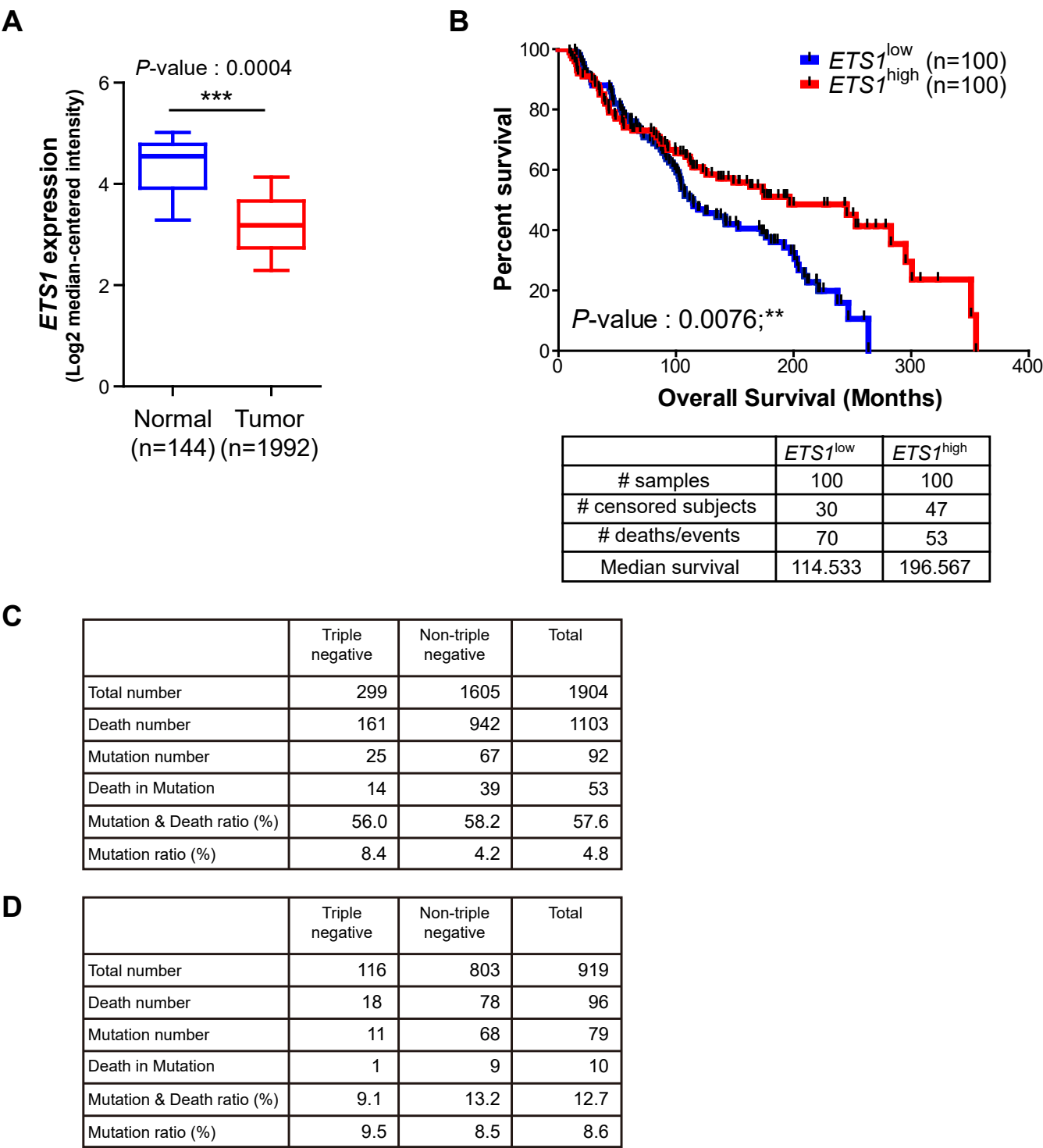
Supplementary Table 4. Differential expressed genes (DEGs) in RNA-seq

Supplementary Table 5. Gene Ontology of DEG

Supplementary Table 6. List of Tumor suppressor genes (TSGs)

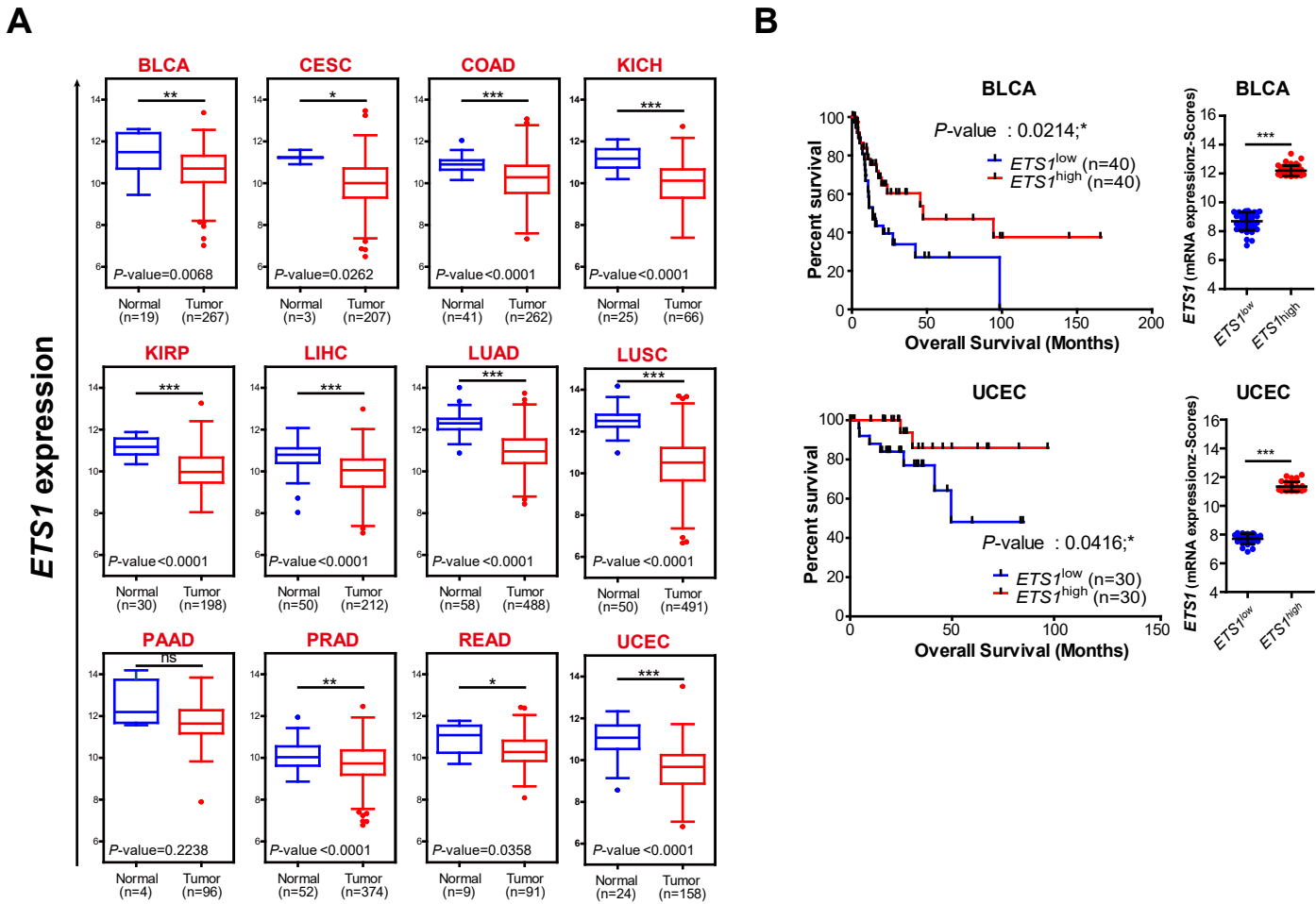
Supplementary Table 7. Overlap DEGs with TSGs

Supplementary Figure 1.



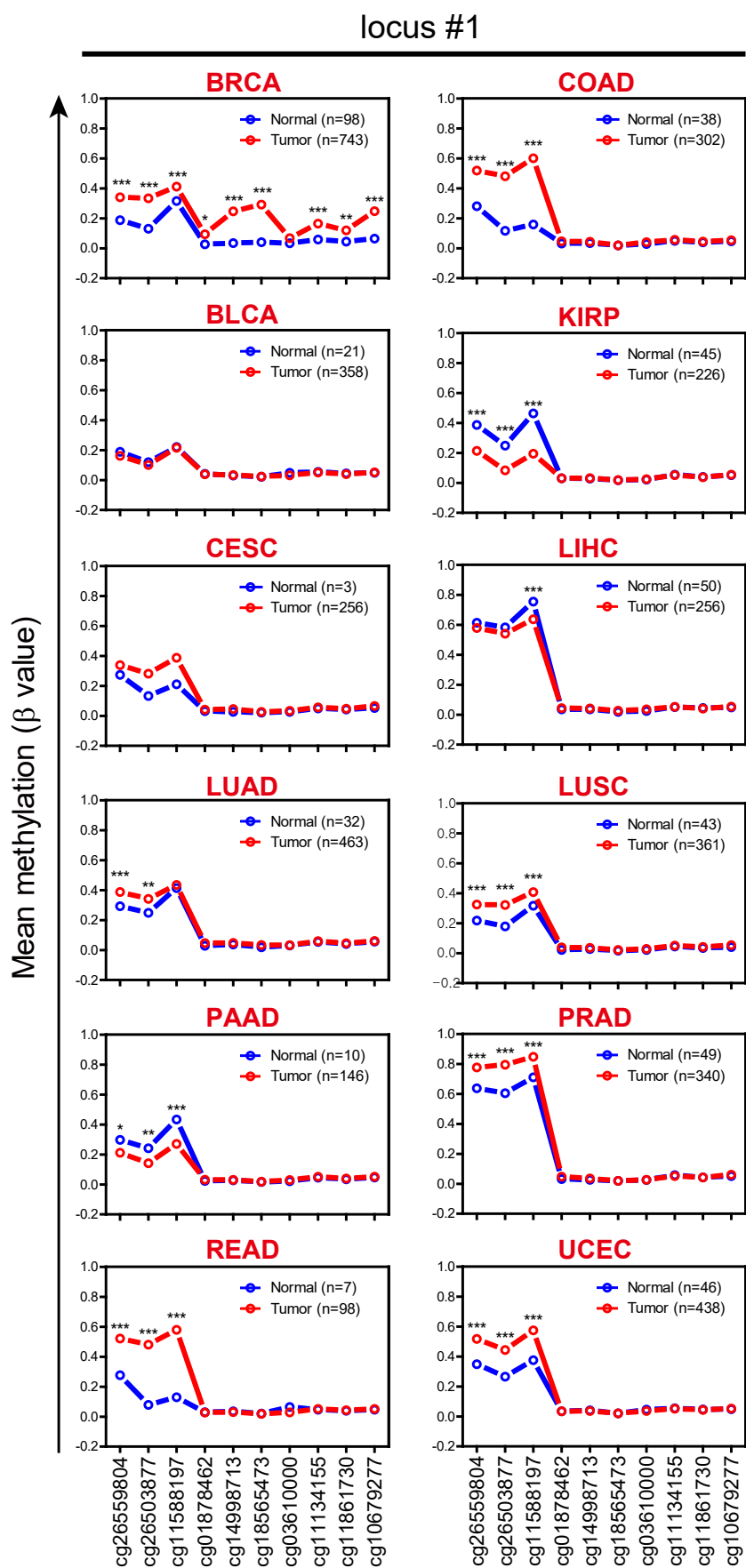
Supplementary Figure 1. Comparison of *ETS1* expression between normal and tumor patient specimens in publically available dataset (Curtis). **(A)** Box plot of *ETS1* gene expression between normal and breast cancer tissues in publically available Curtis data set; normal (n=144) and tumor (n=1992). ****P*<0.001 (Unpaired t test). **(B)** Correlation between *ETS1* expression and survival rate. A total of 1992 samples were divided into *ETS1*^{high} group (n = 100) and *ETS1*^{low} (n = 100) according to *ETS1* expression levels (*ETS1*^{low} or *ETS1*^{high} selection criteria are each less than the lower or higher 10% of the total patient). Overall survival analysis based on *ETS1* levels in BRCA patient. The blue or red lines indicate patients with *ETS1*^{low} (n=100) or *ETS1*^{high} expression (n=100), respectively. *P*-value was calculated with the Log-rank test (*P*-value = 0.0076; **). **(C-D)** Correlation between genetic alteration and survival rate of *ETS1* according to breast cancer subtype in **(C)** Curtis data set and **(D)** TCGA data set.

Supplementary Figure 2.



Supplementary Figure 2. Comparison of *ETS1* expression between normal and tumor patient specimens in diverse cancer types. **(A)** Tumor types that show a reduced *ETS1* expression. BLCA: Bladder Urothelial Carcinoma; CESC: Cervical squamous cell carcinoma and endocervical adenocarcinoma; COAD: colon adenocarcinoma; KICH: Kidney Chromophobe; KIRP: Kidney renal papillary cell carcinoma; LIHC: Liver hepatocellular carcinoma; LUAD: Lung adenocarcinoma; LUSC: Lung squamous cell carcinoma; PAAD: Pancreatic adenocarcinoma; PRAD: Prostate adenocarcinoma; READ: Rectum adenocarcinoma; UCEC: Uterine corpus endometrial carcinoma. * P <0.05, ** P <0.01, *** P <0.001 (Unpaired t test). **(B)** Overall survival analysis based on *ETS1* levels in BLCA, UCEC. The blue or red lines indicate patients with *ETS1*^{low} or *ETS1*^{high} expression, respectively. P-value was calculated with the Log-rank test (* P <0.05) and *** P <0.001 (Unpaired t test).

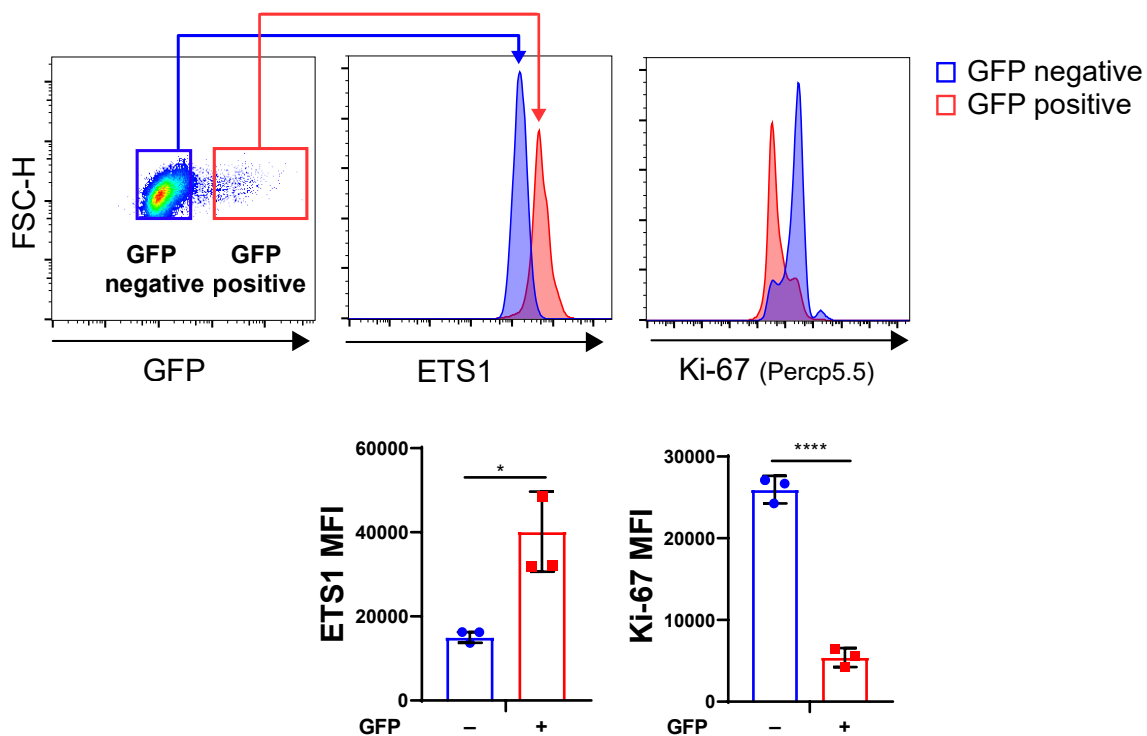
Supplementary Figure 3.



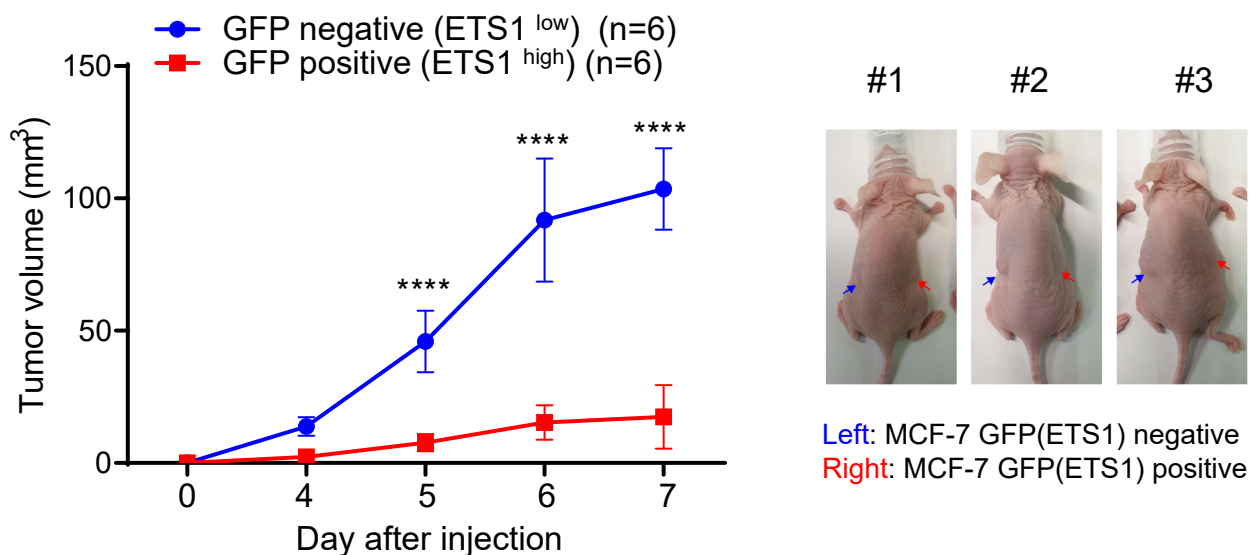
Supplementary Figure 3. DNA methylation status on ETS1 promoter (locus #1) in diverse normal and tumor specimens. Analysis of mean methylation β -value on 10 CpG sites of *ETS1* locus #1 between normal (n=98) and BRCA patient (n=743) specimens by TCGA Wanderer tool. The blue or red line represents normal or BRCA specimen, respectively. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (two-way ANOVA with Bonferroni test).

Supplementary Figure 4.

A



B



Supplementary Figure 4. ETS1 inhibits the proliferation and growth of MCF-7 breast cancer cells *in vitro* as well as *in vivo*. **(A)** MCF-7 cells (ER+, PR+ and HER2-; no ETS1 expression) were transfected with Human cDNA ETS1 GFP vector, and transfected cells were sorted according to GFP -expression. Representative data from three independent experiments. Flow cytometry analysis for ETS1 protein levels and cell proliferation (Ki -67) in GFP negative and positive. *P<0.05, **P<0.01, ***P<0.001 ****P<0.0001 (Unpaired t test). **(B)** Tumor growth curves in nude mice after injection of GFP negative (left) and positive (right) MCF-7 cells. Tumor volumes were measured. Images of representative tumor-bearing mice. Blue arrow: MCF-7 GFP(ETS1) negative; Red arrow: MCF-7 GFP(ETS1) positive. *P<0.05, **P<0.01, ***P<0.001 ****P<0.0001 (two-way ANOVA with Bonferroni test). Data is representative of three independent experiments.

Supplementary Figure 5.

ADAMTS9

TAAATAAAACATGACGCTATCTGGTGCCCCCAATTACTAAGTTACTTTAGTTTGCGGCGTGGG
AGTGGGGATGGGGATATATCTGGCAACAGCAAGGTAGGGGGGGGTTGCCTAAATTCGTTTTCC
ATAGTGTCCTCCCTAGCAATTGGTTGGGGATGACCCAAAGAAGGGAGAGGCTGCAAAGCGG
GAGATAATTTCTTTCTAGGAAAGGAGAGAAAGCCTCCGCTGCGGGGTGCCCTGCCACGAGC
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CCGAGAGCTGAGCCGCTCGGGCCGCAAGGAGGAGCCGGAGGAGCAGGAGGAGGAGGAGC
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TGCACGCCTCTAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG
CCAGACCATGTCCCTCCCTCGGCCGCGCGGGACTCGCAGCCGAGGCCCTGCCGGC
TGCAAGAGGCGGAGGCCAGAGGCGTCACCAGCGCCGGGGCAGCTGTTCTTGTCCTCCCGCCA
CCTCGCTGAAGTGGGGTTTTCTGAGATTCCTGCCCGGGGAGGAGGAGGAGGAGGAGGAGG
CTCCCTTTCTGCGCGCGCTGACGCCACTTCCCTCTCGTCTTTTCTCTCTCTCTCTCTCT
TCTTCTTCCGAGTCTTTTTCT
CTGTCCCTTCTC

Red: ETS1 binding sites
Green: ChIP primer

NOTCH1

CCGCCCCCGCCGGCCCCACCCCCGCCGGTCCCCCGCCCCCTCCGCGGCCACCTCCTCC
CGCACGCCCCGTCCGCCTTTGCGCCTCCCCGAGCTGAGCCGCGCGTCCCAGAGCCAGCCT
CTCCGGGGAGGAGGGACCCGGCGGCGCGGCGTGGGTCCGGAGCGGGCGCTGGGACTAC
TTCTCGTTTGAAGTTTTAGAGGCCAAAAGTTTGAGCCGGGGC|CGGGAAGG|ATCCGGCTC
TGGCGTTGCCGCGCTCGGCGACAACGGCGACTGAAATTTGCATGTGAATAAGCGGAGGA
GCCCCGGGGCGGAATGGGGAGGGCGAGGCCGCGGACAGCCGTGTGCACCGTGCCTGGGC
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TXNIP

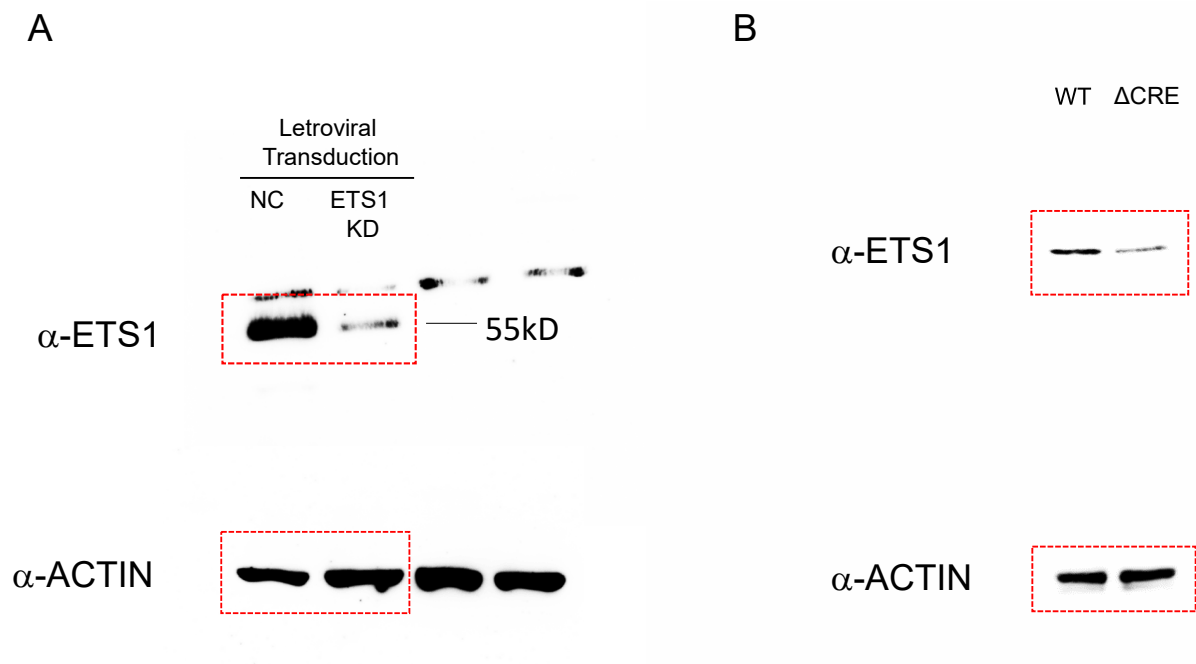
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 TTGGTGGGGCTCCTGGTAAACAAGGACCGGGCAGCCAATGGGAGGGATGTGCACGAGGGCA
 GCACGAGCCTCCGGGCCAGCGCTCGCGTGGCTCTTCTGGCCCCGGGCTACTATATAGAGACG
 TTCCGCTCCAGCTTGAACTAACCCCTCTTTTTCTCCAAAGGAGTGCTTGTGGAGATCGGA
 TCTTTCTCCAGCAATTGGGGGAAAGAAGGCTTTTCTCTGACTTCGCTTAGTGTAACCAGCG
 GCGTATATTTT

STAT5A

ATTTGGGGGTGAAGAGCAGTTAGGGGCAAGGAGGCCAGCCTCGGACCGCAGCTCAGTCAGAC
CCTTGACAGTTTCTGATCTTCCTGAACCCCCATCCCGTGGCCTCAAGTTTCTCCACAGCCT
GGTCCCCAAAATCACCAGGGCACTTTGGTGGGAAATCACCCACACAAGGGGACAAACAAG
GGGACTGAAAGCAAAAAGAAACCCCAAATTTGACATTTCTGGGTAGTTGTGTTATCTGGAGCCA
CGACTACAGTCACCTTTCCCAACCAACTCTGACACCCCTTCCCTCTACTCCCTTGGGTGCCCT
TGTCCTCCTGGGAAAGTTCCATAGCTCCTCCTCCTCCCACTGCATCTCAAAGGGAGAACAG
GCCTGAAGAAATGGAGAGAAACCCAGACA

Supplementary Figure 5. Bioinformatic analysis of promoter regions of representative ETS1 target genes (A) *ADAMTS9*, (B) *NOTCH1*, (C) *TXNIP* and (D) *STAT5A* promoter sequences were analyzed by ECR browser

Supplementary Figure 6.



Supplementary Figure 6. Full Immuno-blots of the experiments shown in **(A)** Figure 3A **(B)** Figure 3C. Uncropped images are labeled as in the main text and red dot square indicate protein bands of interest.